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Chemical Composition and Biotechnological Properties of a Polysaccharide from the Peels and Antioxidative Content from the Pulp of *Passiflora liguralis* Fruits

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A new polysaccharide with a high molecular weight (greater than 1×10^6 Da) was extracted and characterized from the peels of *Passiflora liguralis* (granadilla) fruits. Chemical composition of the biopolymer, performed by using a high pressure anion exchange-pulsed amperometric detector (HPAE-PAD), showed the presence of six different sugar residues: xylose, glucose, galactose, galactosamine, an unknown component, and fucose in the relative ratio of 1:0.5:0.2:0.06:0.05:trace. The optical rotation of this xyloglucan was $[\alpha]_D^{25\ °C} = -186.42$ (concentration of 1.4 mg/mL of H_2O), and the viscosity was dependent on the concentration and pH, showing a maximum value of 1.4 η at a concentration of 3% in distilled water and a maximum value of 7.0 η in citrate buffer solution. Thermogravimetric analysis indicated that this biopolymer was very stable at high temperatures, showing a degradation temperature at 280 °C. The characterization of the polysaccharide was also investigated by spectroscopic methods (^{1}H NMR and IR) pointing out the complexity of this biopolymer and the presence of sugar residues in α-*manno*, α-*gluco-galacto*, and β-*gluco-galacto* configurations. The formation of a biodegradable film using this novel xyloglucan was reported, and the anticytotoxic activity of the polysaccharide was studied in a brine shrimp bioassay. Considerable antioxidant activity (Trolox equivalent antioxidant capacity (TEAC) value of 0.32 μM/mg fresh product) was noted in the lipophilic extracts of *Passiflora liguralis* fruits, indicating, in this fruit, an alternative source of bioactive compounds.

KEYWORDS: ***Passiflora liguralis*; passion fruit; polysaccharide; biological assay; antioxidant activity; biodegradable film.**

INTRODUCTION

The genus *Passiflora* (Passifloraceae) comprises ∼500 species of herbaceous vines or trees distributed mainly in the warm temperate and tropical regions of America and Africa, with a smaller number of species occurring in southeast Asia, India, Malaysia, and Australia (*1*).

Passiflora species have been used as traditional folk medicines in Europe and North America owing to their sedative and antihypertensive properties (*2*−*4*), and a consistent number of these species are present as official drugs in the pharmacopeias of several countries. Few species show antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (*5*), suggesting a potential use as a plant-derived antibiotic. Moreover, antifungal activity (*6*, *7*) and cytotoxicity against P-338 murine leukemia cells (*8*) have been observed. Besides their useful value as sources of newer biological molecules for pharmaceutical applications, some species are grown for their edible fruits and others are grown outdoors in the warmer parts of world or in glasshouses for their exotic flowers.

Previous investigations of several authors report the isolation of a number of compounds such as alkaloids (*9*), phenols (*10*, *11*), glycosyl flavonoids (*12*−*15*), and cyanogenic compounds (*16*−*18*) from some species of *Passiflora*, mainly *Passiflora incarnata* and *Passiflora edulis*, while only sporadic reports are available on other species of *Passiflora*.

Generally, the genus *Passiflora* represents a good source of bioflavonoids (quercetin, apigenin, kaempferol, and so forth)

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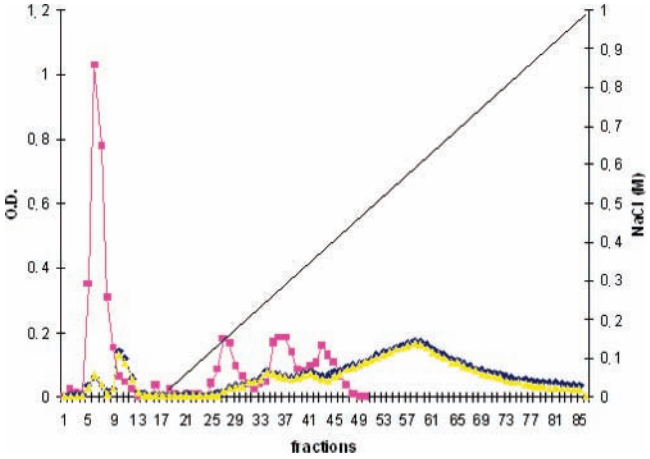

Figure 1. Elution profile from a Sepharose DEAE CL-6B column of crude polysaccharide. [(▲) protein content optical density $\lambda = 280$ nm; (◆) nucleic acid content optical density $\lambda = 260$ nm; (■) carbohydrate content by the Dubois method $\lambda = 490$ nm].

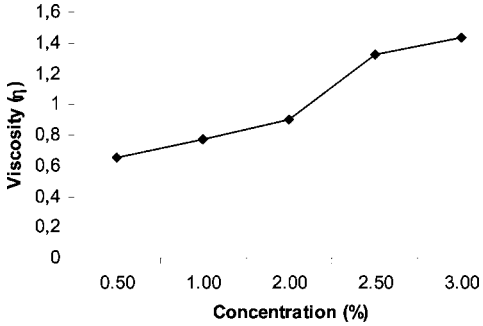

Figure 2. Viscosity/concentration correlation of the *Passiflora* polysaccharide. Specific viscosity (η) measurements as a function of the concentration of an aqueous solution of polysaccharide (0.50%, 1.00%, 2.00%, 2.50%, and 3.00%) were carried out using Cannon-Ubbelohde 75 suspended level viscometers at 30 °C.

which could have therapeutic applications as antioxidants, immunomodulators, and anticarcinogens (*19*−*23*). In addition, a high level of lycopene in the pericarp and in the skin fruits suggested an alternative source of these important nutrients for those people who do not eat tomatoes and tomato products (*24*).

The fruits of some species of *Passiflora*, such as *P. edulis* and *P. liguralis*, beyond their use in the home in fruit salads and in beverages, can be processed industrially to produce tropical fruit juices. Since commercial processing of fruits yields about 30% juice, 52% peels, and 11% seeds, it seems clear that the disposal of fruit waste represents a considerable problem for the producer countries of *Passiflora* juice (*25*).

To obtain products with high added value from *Passiflora* fruit, our studies have concerned the extraction and characterization of a novel polysaccharide from the peels of *Passiflora liguralis* (granadilla) fruits. Biotechnological applications of this biopolymer were investigated. Moreover, we have tested the protective action of the polysaccharide toward avarol, a natural cytotoxic compound isolated from marine sponge, and the antioxidant activity of lipophilic extracts of *Passiflora liguralis* fruits.

MATERIALS AND METHODS

Chemicals. Analytical grade methanol, dichloromethane, diethyl-ether, and ethanol were obtained from Carlo Erba (Italy). *N*,*N*-Dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the crystallized diammonium salt were from Fluka, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Aldrich, and potassium persulfate ($K_2S_2O_8$) was purchased from Sigma Chemical Co. (Italy).

Sampling. The fruits of *P. liguralis* were grown in Perù in better growth conditions: subtropical weather, a temperature of 15−20 °C, and an altitude 1800−2200 m over the sea. The fruits were harvested at the end of April at the peak of their ripening.

Samples were brought to Italy and kept in the lab at −20 °C until analysis. Five fruits (∼600 g) were peeled, and then the pulp of the fruits was removed. The peels (230 g, wet weight) were used for procedures to extract the polysaccharide, and the pulp was submitted to analysis for measurements of antioxidant activity.

Polysaccharide Extraction. The peels of the *P. liguralis* fruits were treated with 500 mL of 5 N KOH under stirring for 3 days at room temperature and then centrifuged at 10 000*g* for 40 min. After centrifugation, the supernatant was precipitated with cold ethanol (v/v) and then stored at −20 °C overnight. The pellet, collected after centrifugation (10 000*g* for 40 min), was dissolved in hot distilled water, dialyzed against running water for 3 days, and then lyophilized under vacuum. The lyophilized sample (2 g) was utilized for further analyses.

Chemical−Physical Analyses. Carbohydrate content was determined according to Dubois's method using glucose as the standard (*26*). Total protein content and nucleic acid content were detected as described by Nicolaus et al. (*27*). Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen's method (*28*). Purification of the polysaccharide was performed by gel filtration on a Sepharose DEAE CL-6B column eluted with 0.1 L of H_2O and 0.4 L of a gradient of NaCl from 0 to 1 M at flow rate of 0.3 mL/min, and fractions of 5 mL were collected. All fractions were tested by using spot on thin-layer chromatography (TLC) involving an α-naphtol spray to identify fractions containing carbohydrates. The molecular weights of the samples (at a concentration of 1 mg/mL in H_2O) were estimated by two methods. The first method was gel filtration on a Sepharose CL-6B column (1 × 80 cm) using H_2O as the eluent with a flow rate of 0.3 mL/min. Fractions of 1 mL each were collected. All fractions were tested by using the α-naphtol spot test as described above. The second method was density gradient centrifugation (*27*) with a sucrose gradient from 0 to 50% (w/v) at 130 000g for 16 h. The centrifuge tubes were fractionated in 0.2 mL fractions diluted with water, dialyzed against water for 72 h, and then tested for the presence of carbohydrate as reported above. In both experiments, 10 mg of polysaccharide and a mixture of dextrans for the calibration curves (2 mg each of the dextran standards: 150 000 Da; 670 000 Da; and 2 000 000 Da) were used.

Hydrolysis of the polysaccharide was performed with 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h. The sugar components were identified at first by TLC using monosaccharide standards for identification. TLC was developed with the following solvent: acetone/butanol/ H_2O (8:1:1, by volume). The sugars were visualized by reaction with α-naphtol. Afterward, the monosaccharide composition was established by using a high pressure anion exchange-pulsed amperometric detector (HPAE-PAD DIONEX) equipped with a Carbopac PA1 column, and the sugars were eluted isotonically with 16 mM NaOH.

The optical rotation value was obtained on a Perkin-Elmer 243 B polarimeter at 25 °C in water.

The NMR spectra of the polysaccharide (15 mg/mL D_2O) were recorded on a Bruker AMX-500 MHz spectrometer at 70 °C. Chemical shifts were reported in parts per million (ppm) relative to sodium 2,2,3,3-d4-(trimethylsilyl) propanoate for ^{1}H NMR (*29*). Samples were exchanged twice with D_2O with an intermediate lyophilization step and then dissolved in 500 μL D_2O.

Fourier transform infrared (FTIR) spectra were recorded by using a Perkin-Elmer Paragon 500 single-beam spectrophotometer. The sample as a powder was ground with KBr and put under the beam as a disk, and then the spectrum was collected after 16 scans under nitrogen.

Measurements of the specific viscosity (η) as function of the concentration of an aqueous solution of polysaccharide were car-

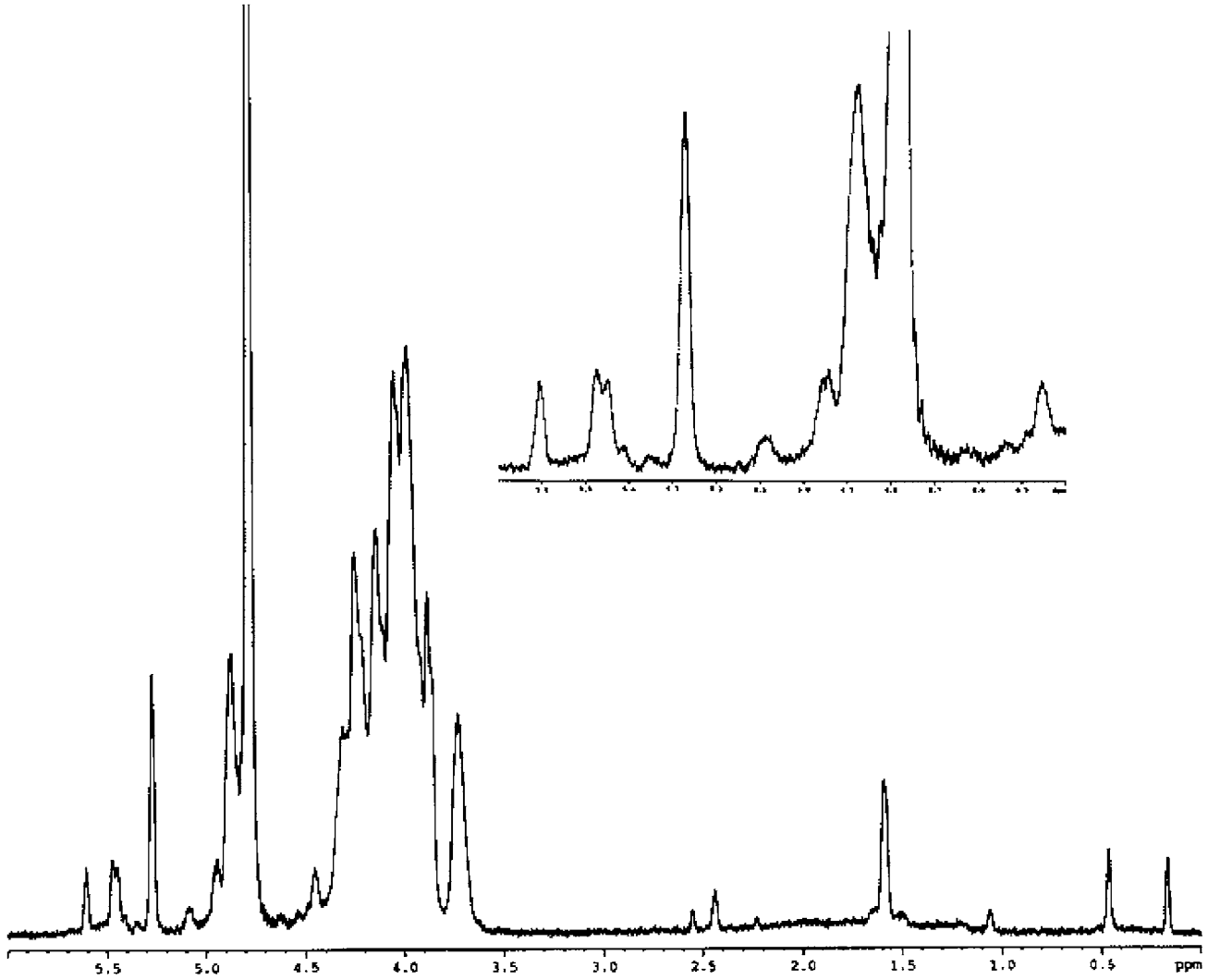

Figure 3. ^{1}H NMR spectrum of the *Passiflora* polysaccharide. NMR spectra of the polysaccharide (15 mg/mL D_2O) were recorded on a Bruker AMX-500 MHz spectrometer at 70 °C. Chemical shifts were reported in parts per million (ppm) relative to sodium 2,2,3,3-d4-(trimethylsilyl) propanoate. The sample was exchanged twice with D_2O with an intermediate lyophilization step and then dissolved in 500 μL of D_2O. In the inset is reported an amplified anomeric region (from δ 4.5 to δ 5.6 ppm).

ried out using Cannon-Ubbelohde 75 suspended level viscometers at 30 °C. The specific viscosity was calculated by applying the following formula:

$$(t - t_0/t_0)/C$$

where t is the time (s) employed from the polysaccharide solution to cover an established distance in the viscometer, t_0 is the time (s) employed from the distilled water to cover an established distance in the viscometer, and C is the concentration (%) of the polysaccharide solution.

The rheological properties were characterized by studying the specific viscosity (η) of the samples (at a concentration of 1%) with respect to the pH range (2.32−8.36) by using 50 mM citrate-phosphate buffer at 30 °C.

Biological Assay. Avarol, used for the brine shrimp test, is a natural sesquiterpene hydroquinone isolated from the *Dysidea avara* sponge (*30*). The brine shrimp (*Artemia salina*) assay was performed in triplicate with the appropriate amounts of polysaccharide dissolved in DMSO (1% final volume) at concentrations of 500, 50, and 5 ppm, in the presence of avarol at 10 ppm, using 10 freshly hatched larvae suspended in 5 mL of artificial seawater. Briefly, for each dose tested, the surviving shrimps were counted after 24 h, and the data were statistically analyzed by using the Finney program, which affords LD_{50} values with 95% confidence intervals (*31*).

Thermogravimetrical Analysis. Thermogravimetrical analysis (TGA) was performed by using a Mettler TGA apparatus. Samples (5 mg) were heated from 30 to 400 °C at a heating rate of 20 °C/min under nitrogen.

Biodegradable Film. Biodegradable films were formed by solubilizing 50 mg of polysaccharide in 5 mL of water at room temperature. After dissolution, 5 mg of glycerol was added as plasticizer. The solution was poured into a Petri dish to allow the evaporation of water. The biofilms were analyzed with the dynanometer test. Stress was applied by using a tensile dynanometer machine by Instron.

Antioxidant Activity. The pulp of granadilla (163 g) was homogenized in a blender with distilled water (163 mL), yielding 340 mL of homogenate. The total homogenate was centrifuged at 10 000*g* for 40 min, and the supernatant (156 mL) and pellet (95.1 g) were collected separately and then immediately analyzed.

The supernatant collected was tested for hydrophilic antioxidant activity, carried out in triplicate according to the DMPD method developed by Fogliano et al. (*32*), and antioxidant activity was quantified in terms of Trolox equivalent antioxidant capacity (TEAC, μM).

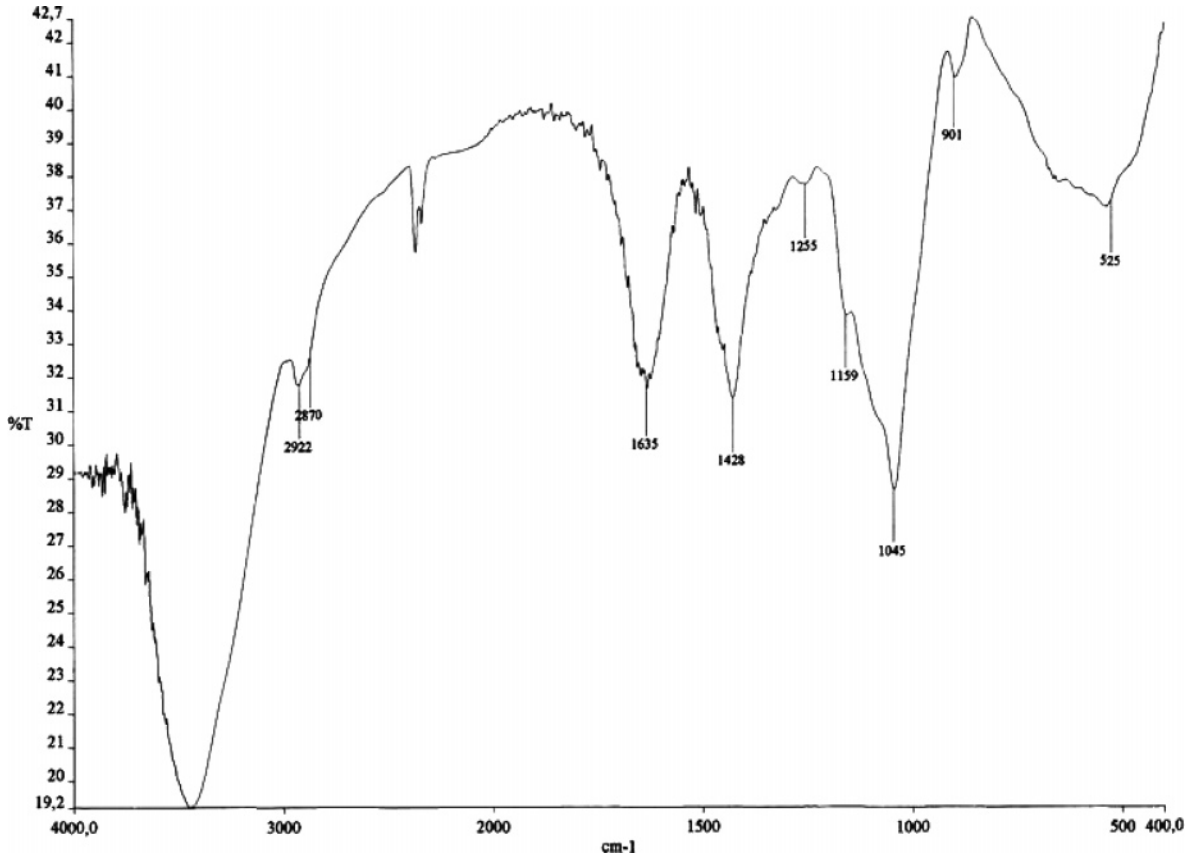

Figure 4. IR spectrum of the *Passiflora* polysaccharide. The FTIR spectrum was recorded by using a Perkin-Elmer Paragon 500 single-beam spectrophotometer. The sample as a powder was ground with KBr and put under the beam as a disk, and then the spectrum was collected after 16 scans under nitrogen.

Table 1. Chemical Shifts and Coupling Constants of Anomeric Signals in the ^{1}H NMR Spectra of a *Passiflora* Polysaccharide Recorded on a Bruker AMX-500 MHz Spectrometer at 70 °C

residues	δ (ppm)	J (Hz)	absolute configuration
A	4.87 (bs)	<1.0	α-*manno*
B	4.94 (d)	5.7	α-*gluco-galacto*
C	5.08 (s)		
D	5.27 (s)		
E	5.47 (d)	10.3	β-*gluco-galacto*
F	5.60 (s)		

The pellet was extracted with diethyl ether (1:2, w/v) under stirring in the dark overnight. The lipophilic extract was filtered, concentrated in a rotary evaporator in vacuum (T < 35 °C), and then dried under N_2 flux.

The dried diethyl ether extract (3.87 g) was redissolved in 10 mL of CH_2Cl_2 (analytical grade), and 5 μL of this solution was tested.

The lipophilic antioxidant activity assay was carried out in triplicate according to the ABTS method as described by Miller et al. (*33*) and Miller and Rice-Evans (*34*) and then quantified in terms of TEAC.

RESULTS AND DISCUSSION

Polysaccharide Extraction and Chemical Studies. A new polysaccharide was extracted and characterized from the peels of *Passiflora liguralis* fruits using a simple and rapid method with low environmental impact.

Starting from 230 g of wet peels, we have obtained 2 g of lyophilized biopolymer with high carbohydrate content (75.5%), low protein (1%) and nucleic acid (1%) concentrations, minor compounds (acetyl and sulfate groups 11%), an unknown (5%), and the presence of uronic acids of 5.5% in contrast to that of the cell-wall pectic polysaccharides that contain arabinose in large amounts and a higher content of uronic acids (*35*). These unusual findings are due to different minor polysaccharides extracted by the use of our extraction method.

Raw polysaccharide material (35 mg) was purified by chromatography on a Sepharose DEAE CL-6B column eluted with 0.1 L of H_2O and 0.4 L of a linear gradient of NaCl from 0 to 1 M with a yield of 28%. The elution profile is shown in **Figure 1**.

Three sugar fractions were obtained: fraction 1 (9.7 mg) was eluted only in water, fraction 2 (11.4 mg) was eluted in 0.1 M NaCl, and fraction 3 (10 mg) was eluted in 0.25 M NaCl. The three fractions were tested for carbohydrate content: only the carbohydrate content of fraction 1 resulted in a very high value (75%), while fractions 2 and 3 had 21.5% and 6.5% carbohydrate content, respectively. Since fraction 1 showed the highest carbohydrate content while the protein and nucleic acid content was less than 1%, all the following analyses were performed only on this fraction.

The molecular weight of fraction 1 was estimated from density gradient centrifugation using a sucrose gradient and from a calibration curve of standard dextrans obtained by gel filtration on a Sepharose CL-6B column; it was approximately higher than 1×10^6 Da.

The optical rotation of the polysaccharide was $[\alpha]_D^{25\ °C} = -186.42$ (concentration of 1.4 mg/mL of H_2O).

Measurements of viscosity (η) were performed using different concentrations of an aqueous solution of polysaccharide (0.5%, 1%, 2%, 2.5%, and 3% w/v). We observed a linear correlation between the concentration and the viscosity of the polysaccharide solution, with a lower viscosity (0.63 η) at a concentration

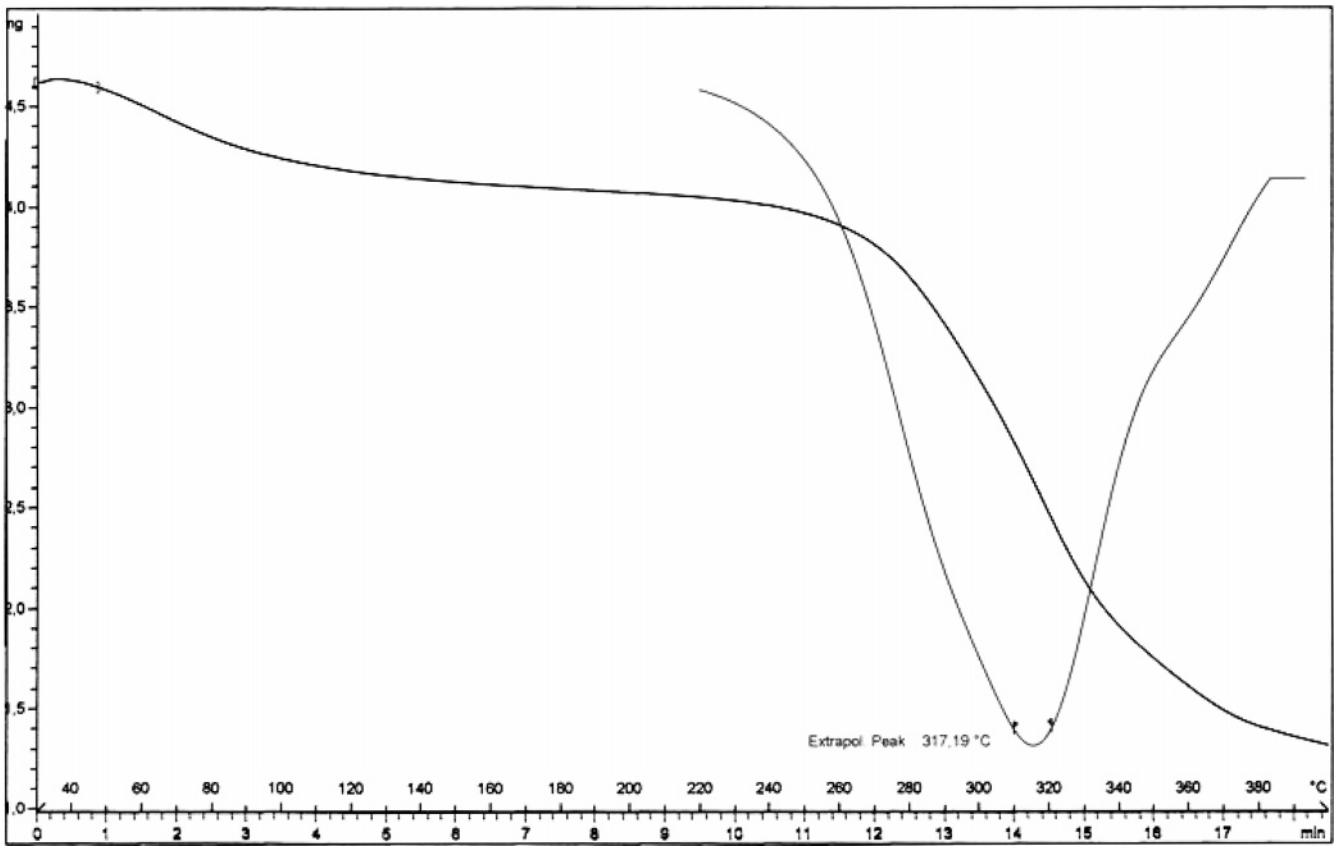

Figure 5. Thermogravimetrical analysis of the polysaccharide. TGA was performed by using a Mettler TGA apparatus. The sample (5 mg) was heated from 30 to 400 °C at a heating rate of 20 °C/min under nitrogen.

of 0.5% and a higher viscosity (1.4 η) at the maximum concentration (3%) (**Figure 2**). Also for measurements of the viscosity in a pH range (2.32−8.36), there was a linear increase of the viscosity in relation to an increase of the pH. What was very interesting is, instead, the great difference in the viscosity between the aqueous solution and buffer solution of polysaccharide. We observed an increase of the viscosity from a range of 0.63−1.4 η in aqueous solution to 6.0−7.0 η in citrate-phosphate buffer solution.

After hydrolysis of the sample with 2 M TFA, the sugar components, identified by both TLC and HPAE-PAD Dionex, were as follows: xylose, glucose, galactose, galactosamine, an unknown component, and fucose in the relative proportion of 1:0.5:0.2:0.06:0.05:trace. The polysaccharide obtained from *Passiflora liguralis* peels was a biopolymer with a xyloglucan-like structure with interesting chemical−physical properties such as high viscosity and high molecular weight.

The ^{1}H NMR spectrum of the polysaccharide (**Figure 3**) showed a complex profile. It exhibited six well resolved peaks in the anomeric region (from δ 4.5 to δ 5.6 ppm; δ chemical shifts are expressed in ppm) at δ 4.87 (broad singlet, bs, $J <$ 1.0 Hz); δ 4.94 (d, $J = 5.7$ Hz); δ 5.08 (s); δ 5.27 (s); δ 5.47 (d, $J = 10.3$ Hz); δ 5.60 (s). The signals in the upfield region of the spectrum indicated the presence of deoxy-sugars (δ 1.59) and acetamino sugars (δ 2.45−2.55). The remaining signals (δ 3.5−4.5) were due to ring protons, confirming the presence of pyrosidic exose.

The six anomeric signals indicated the presence of six different monosaccharides, that are present in the repeating unit, in relation to type or glycosidic linkage position. Those six residues are shown in **Table 1** from A to F as described with respect to increasing δ. Chemical shifts and coupling constant values indicate that residue A has probably an α-*manno* configuration, residue B has an α-*gluco-galacto* configuration, while residue E has a *β-gluco-galacto* configuration.

The IR spectrum (400−4000 cm^{-1}) of the polysaccharide is reported in **Figure 4**. It shows several characteristic bands typical of a polysaccharide structure that can be attributed to the presence of functional groups in the biopolymer. The spectrum presented bands at 3000−3600 cm^{-1} of an O−H stretching vibration and at 2870−2922 cm^{-1} of CH_2 asymmetric and symmetric stretching vibrations. The band at 1635 cm^{-1} could be attributed to a *N*-acetyl group of some sugar residue or to the stretching vibration of C═O. A broad absorption band attributable to S═O was observed at 1240 cm^{-1}. Bands at 1159 and 1045 cm^{-1} could be attributed to the stretching vibrations of C−O−C and O−H, respectively. The absorption occurring at ∼900 cm $^{-1}$ shows the β-configuration of the glucan linkages of some residues.

Biological Assay. We have studied the effect of a polysaccharide from the peels of *Passiflora liguralis* fruits on the inhibition of cytotoxic effects produced by avarol.

The anticytotoxic activity of the polysaccharide to induce the inhibition of avarol (10 μg/mL) toxicity in a brine shrimp (*Artemia salina*) bioassay was evaluated. Avarol (a natural toxic sesquiterpene hydroquinone isolated from the *Dysidea avara* sponge) shows strong toxicity ($LC_{50} = 0.18$ μg/mL) in the brine shrimp bioassay, which gives results that correlate well with those for cytotoxicity in cancer cell lines such as KB, P388, L5178y, and L1210 (*36*). For this assay, we selected a concentration of avarol of 10 ppm to obtain total brine shrimp larvae death. The new biopolymer isolated from *Passiflora* peels was found to be an anticytotoxic compound in the brine shrimp

Table 2. Polysaccharide Inhibition of Avarol Toxic Activity on *Artemia salina* (Brine Shrimp Bioassay)

	500 ppm[a]	50 ppm[a]	5 ppm[a]	LD_{50} (ppm)
Passiflora polysaccharide	28/30[b]	26/30	28/30	na
Passiflora polysaccharide + 10 ppm avarol	10/30	0/30	0/30	2.1268(4.8563/0.0845)[c]

[a] Concentration of polysaccharide. [b] Survivals/total larvae of *Artemia salina.* [c] Values of 95% confidence intervals; na = no activity.

bioassay, increasing the LD_{50} value of avarol from 0.18 up to 2.1268 μg/mL when present at a concentration of 500 ppm (**Table 2**).

Thermogravimetrical Analysis and Biofilm Formation. The results for thermogravimetrical analysis are reported in **Figure 5**. The polysaccharide showed a weight loss due to water presence, centered at 60−80 °C. From this temperature to ∼260 °C, the biopolymer was very stable and started to decompose at ∼280 °C, indicating a behavior similar to that of other polysaccharides, such as alginates and chitosan.

A useful application of this polysaccharide could be the formation of biodegradable films using these biopolymers upon addition of glycerol. Applying this procedure, we obtained a solid, clear, and elastic film able to recover small deformations produced by the applied tensile stress. These biofilms could have an interesting biotechnological application and could be used in different fields such as agriculture, that is, for protected cultivation with a mulching operation technique.

Antioxidant Activity. A comparative study of the antioxidant activity between the hydrophilic and lipophilic portions of the homogenate of the *Passiflora liguralis* pulp, using DMPD and ABTS assays, respectively, revealed that the hydrophilic fraction (obtained after the homogenization of 160 g of pulp fruits) showed a TEAC value corrisponding to 18.1 μM, while the lipophilic fraction (3.87 g/10 mL of CH_2Cl_2) presented a TEAC value of 26 μM. These data showed that the results obtained from the lipophilic fractions of *Passiflora* fruits are comparable with those obtained from tomato (*37*).

Conclusion. The xyloglucan biopolymer obtained from *Passiflora liguralis* peels had good qualities for possible biotechnological employment due to its high molecular weight and high viscosity. The method described in this paper is rapid and easy with low environmental impact and is useful for the extraction of a polysaccharide from the peels of *Passiflora liguralis* fruits.

Passiflora fruits can be processed industrially to achieve tropical fruit juice. The disposal of fruit waste represents a considerable problem for the producer countries of *Passiflora* juice. It is the general opinion that new biotechnology knowledge can be applied to manipulate these residues, transforming them to a source of natural products, such as polysaccharides (*38*), with interesting properties. These biopolymers are unique for their chemical−physical properties (emulsifying, viscoelasticity, polyelectrolyte, adherence, biocompatible, stabilizer, and so forth), and they have the ability to interact with other polymers, such as proteins, lipids, as well as polysaccharides (*39*). The *Passiflora* polysaccharide, for its chemical−physical characteristics, showed the ability to form a film when added to glycerol in defined conditions. Moreover, thermogravimetrical analysis indicated that this xyloglucan was very stable with a high decomposition temperature (280 °C) similar to that of other polysaccharides. The extraction method reported here has also been used successfully with the solid waste of the tomato-processing industry (*40*) and may be extended to other types of food packaging discards with the aim to obtain products with high added value.

By using the brine shrimp bioassay, it was demonstrated that this biopolymer is a bioactive compound, in that it is able to induce inhibition of avarol toxicity against biological systems, increasing the avarol LD_{50} value from 0.18 up to 2.1268 μg/mL.

In the antioxidant activity test, the results obtained from the lipophilic fraction of the *Passiflora* fruit pulp are comparable with those obtained from tomato. It is known that tomato is a rich source of antioxidant products, particularly carotenoids (lycopene and β-carotene). In our previous studies, we reported the different antioxidant activities of nine different cultivars of tomato (*37*), showing that the best antioxidant activity for a lipophilic fraction was measured for the San Marzano cultivar, as a TEAC value of 0.55 μM referred to 1 mg of fresh product. The lipophilic fraction of *P. liguralis* pulp showed that a TEAC value of 0.32 μM referred to 1 mg of fresh product. These results are probably due to the high levels of lycopene in *Passiflora liguralis* fruits, making them an alternative source of important nutrients, such as lycopene, for those people who do not eat tomatoes and tomato products.

Our study, regarding the possibility to obtain a new polysaccharide from *Passiflora liguralis* with biological activities and biotechnological properties, could provide an useful way to recover bioactive compounds from discards deriving from the *Passiflora* juice producer industry.

ABBREVIATIONS USED

ABTS, 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DEAE, diethylaminoethyl; HPAE-PAD, high pressure anion exchange-pulsed amperometric detector; TEAC, Trolox equivalent antioxidant capacity.

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